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(54) Title: TREATMENT OF PATHOLOGICAL CONDITIONS INFLUENCED BY THE ACTION OF MATRIX METALLOPROTEINASES (MMPs) USING CLIOQUINOL

(57) Abstract: A use of clioquinol for the manufacture of a pharmaceutical composition for the prevention or the treatment of pathological conditions influenced by the action of matrix metalloproteinases (MMPs) is disclosed. Also methods of treatment or prevention of such conditions are disclosed.

TREATMENT OF PATHOLOGICAL CONDITIONS INFLUENCED BY THE ACTION OF MATRIX METALLOPROTEINASES (MMPs) USING CLIOQUINOL

Introduction

The present invention relates to a new use of the known compound clioquinol. Especially, the invention pertain to the use of clioquinol for the manufacture of a pharmaceutical composition for treatment or prevention of pathological conditions influenced by the action of matrix metalloproteinase (MMP).

Background of the invention

A group of enzymes involved in the breakdown of various biological substances is generally known as matrix metalloproteinases, referred to herein as MMPs. The group of MMPs comprises at least 13 different enzymes, including stromelysin, gelatinase, and metalloelastinase.

The common characteristic of the enzymes of the MMP group is that they require and are dependent on the presence of Zn^{2+} to be active, as the structure of MMPs show the presence of a zinc(II) ionic site associated with the catalytic site.

The function of MMPs in the body is to degrade extracellular proteinious matrix components. MMPs degrade collagen, laminin, proteoglycans, fibronectin, elastin, gelatin, myelin etc. under physiological conditions. The normal action of MMPs is *inter alia* effective on tissue remodeling of articulation tissue, bone tissue and connective tissue. The homeostasis of the extracellular matrix is controlled by a delicate balance between the synthesis and activation of MMPs, the degradation of MMPs, and the presence of MMP inhibitors.

It is generally accepted that a derivation from the normal overall level of the MMPs and the proportion between the individual MMPs may play a role in pathological conditions involving tissue breakdown, e.g. rheumatoid arthritis; osteoarthritis; osteopenias such as osteoporosis, periodontitis, gingivitis, corneal epidermal or gastric ulceration; and tumour metastasis, invasion and growth. MMPs are also expected to be responsible, at least in part, for the development of neuroinflammatory disorders, including those involving myelin degradation, e.g. multiple sclerosis, as well as for the management of angiogenesis dependent diseases, which include arthritic conditions and solid tumour growth as well as psoriasis, proliferative retino-pathies, neovascular glaucoma, ocular tumours, angiofibromas and hemangiomas. However, the relative contribution of individual MMPs in any of the above disease states is not yet fully understood.

Modulation of MMP regulation is possible at several biochemical sites but direct inhibition of enzyme action provides a particularly attractive target to therapeutic intervention. *In vivo,* the MMPs are regulated by tissue inhibitors of metallopro-teinases (TIMPs).

The present invention is directed to a synthetic compound having the property of inhibiting the action of MMPs. Thus, the compound is useful in the treatment or the prophylaxis of the above pathological conditions.

Prior Art

The involvement of inhibitors of MMPs in cancer has been the subject of continuous scientific interest for at least 10 years and investigations have pointed

not only to a role of inhibitors of MMPs in invasion and metastasis but also in tumour growth, apoptosis, transformation, and angiogenesis. The inhibitors of MMPs cannot only block tumor invasion and metastasis but also inhibit the growth of primary tumors. As an example, leukemia cells secrete in tissue culture MMPs, one of which is the known MMP-9. It has been shown that chemical chelators, such as EDTA and phenanthrolene, are able to inhibit the activity of said MMPs and halt the degradation of the matrix constituents (Dittman KH et al., Exp Hematol 23:155, 1995).

The balance between activation of MMPs and their inhibition is a crucial aspect of cancer invasion and metastasis. In colorectal, breast, prostate and bladder cancer, most patients with aggressive diseases have increased plasma levels of gelatinase B (Zucker S et al., Ann NY Acad Sci 878:212, 1999). The role of MMPs in tumour angiogenesis and growth is established in both human and animal experimental models wherein there is a necessity for the degradation of the stromal matrix during the neoplastic process and, either directly or indirectly, the tumour is able to achieve this via MMP action.

Both type I and type II diabetes complications (kidney, eye, peridontal) are likely to be improved by the administration of inhibitors of MMPs. Tetracycline analogues that inhibit MMPs have been evaluated experimentally (Ryan ME et al., Ann NY Acad Sci 878:311, 1999). Their results have shown a reduction in the incidence of cataract development, proteinuria and tooth loss. It is proposed that one of the mechanisms of action of inhibitors of MMPs in peridontal disease, irrelevant of diabetes complications, is the inhibition of elevated levels of

MMPs, including neutrophil and bone cell collagenases (MMP-8 and -13) which are associated with the host response in chronic adult periodontitis (Ashley RA et al., Ann NY Acad Sci 878:335, 1999).

It is known that articular cartilage is composed of an abundant extracellular matrix that is rich in collagen and sulfated proteoglycans. The contents of proteoglycans within the collagen network provide cartilage with compressibility and elasticity necessary to protect and cushion the subchondrial bone. During the development of osteoarthritis, the physical characteristics of the cartilage matrix become disrupted and a loss of collagen and proteoglycan from cartilage occurs, which is the hallmark of the disease (Leff RL Ann NY Acad Sci 878:201, 1999). In both osteoarthritis and rheumatoid arthritis as well as in other arthritis and fibrosis, the MMPs have been disclosed as implicated. A variety of cell types, including chondrocytes and synoviocytes, secretes the MMPs, and the progress of diseases is associated with an increase in the concentrations of MMPs in plasma and synovial fluid. Inhibition of the activity of such degenerative enzymes may halt or slow the progression of osteoarthritis and the other arthritis and fibrosis conditions and ameliorate the course of the diseases. In both human rheumatoid arthritis (Ahrens D et al., Arthritis Rheum 39: 1576, 1996) and in experimental animal uveitis (Di Girolamo N et al., Curr Eye Res 15:1060, 1996) there is an increased expression of MMPs (MMP-9, -1, and -3, respectively).

The generalised loss of bone, the development of osteoporosis, and the subsequent occurrence of fractures all increase with age. Oestrogens deficiency leads to an increase in bone resorption, probably

secondary to an increase in osteoblast number and collagenase activity. It has been shown (Williams S et al., Ann NY Acad Sci 878:191, 1999) that minocycline, a collagenase inhibitor, changes the spectrum of bone remodeling and throughout this activity favours bone formation.

Some members of the MMP family are active in vascular matrix remodeling in the pathogenesis of atherosclerosis. It seems that said MMPs may be over expressed in certain locations of atherosclerotic plaques and contribute to the destruction of connective tissue and thus plaque rupture. In the majority of lesion areas, however, matrix synthesis is likely to outstrip matrix degradation, because accumulation is a major feature of most atheromas. MMPs expressed in atherosclerosis are the matrix metalloproteinases-3 (stromelysin), -9, -12, and -13. This type of imbalance favouring matrix deposition is likely to be exacerbated in individuals with the 6A6A genotype in whom stromelysis expression is lower due to the weaker stromelysin promoter.

Acute coronary syndromes result from fissure, erosion or rupture of a vulnerable atherosclerotic plaque. The characteristics of a vulnerable plaque include a large lipid pool, an abundance of inflammatory cells and mediators, a reduced smooth muscle cell and collagen content and a thin overlying fibrous cap. There is evidence supporting that the plaque stabilisation may be achieved through inhibition of MMPs.

Matrix synthesis and degradation contribute to the morphological changes occurring after a myocardial infarction. Mast cells appear to play an important role in the destabilisation of the atherosclerotic plaque. Said instability is associated with increased

numbers of mast cells in culpit lesions. Activated mast cells secrete neutral proteases capable of degradation of the extracellular matrix by stimulating macrophages to produce MMP-9. It has been shown that administration of an inhibitor of MMPs attenuates early left ventricular models.

In the normal heart, cardiomyocytes are surrounded by extracellular matrix and latent MMPs produced primarily by cardiac fibroblasts. The development of congestive heart failure is associated with ventricular dilation and myocardial remodeling. It has been shown that the contributory mechanism for the initiation of the dilation remodeling is enhanced expression and potentially increased activity of left ventricular MMPs (Spinale FG et al., Circ Res 82:482, 1998). This may lead to activation of adverse MMPs remodeling, cardiac dilatation and cardiac failure.

Changes in copper concentration in the arterial wall are important because of cross-linkage formation in collagen and elastin. In a study undertaken to evaluate the concentrations of heavy metals in arterial wall, serum and calcified atherosclerotic plaques showed an accumulation of Ca, Mg, Zn and Cu atherosclerotic plaques (Iskra M et al., J Trace Elem Med Biol 11:248, 1997).

It has been shown that EDTA, 1,10-phenanthroline as well as inhibitors of MMPs reduce the activities of MMPs that dysregulate extracellular matrix and contribute to vascular remodeling as complications of atherosclerotic lesions (Galis ZS et al, J Clin Invest 94:2493, 1994).

The abdominal aortic aneurysms represent a chronic degenerative condition associated with a life-threatening risk of rupture. The condition is thought to be due to a progressive degeneration of the aortic

wall elastin and collagen and in the increased production locally of MMPs. It has been shown that even short term treatment experimentally with inhibitors of MMPs suppress the expression of MPPs in the aortic tissue.

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) was previously frequently used for the treatment of various disorders, such as amoebiasis and non-specific infectious diarrhea (Kono, 1975, Japan J. Med. Sci. Biol., 28: 1-19, Meade, 1975, Brit. J. prev. soc. Med., 29: 157-169). However, the use of clioquinol was stopped due to the presumption that clioquinol caused subacute myelo-optico-neuropathy (SMON).

Renewed interest has been evinced in clioquinol recently as it has been shown to be effective in the treatment of Heliocobacter pylori (WO 95/31199) and neurotoxic injury (WO 97/09976). Furthermore, in US 5,980,914, clioquinol has been suggested for the treatment of Parkinson's disease and in WO 98/06403, clioquinol has been suggested for the treatment of Alzheimer's disease. In WO 99/34807 it is stated that the hydrophobic binding of vitamin B_{12} to a metabolite of clioquinol (clioquinol glucuronide) is believed to cause the vitamin B_{12} to be excreted from the body together with clioquinol glucuronide, thus preventing resorption of vitamin B_{12}, which would eventually lead to a vitamin B_{12} deficiency. Therefore, vitamin B_{12} deficiency is believed to be, at least to some extent, the underlying cause of SMON.

Phanquinone (4,7-phenanthroline-5,6-dione) has hitherto been used for the treatment of various disorders, such as amoebiasis. Phanquinone has been sold by CIBA-GEIGY under the trademark ENTOBEX. In contrast to clioquinol no adverse side effects have

been detected when phanquinone is used in the normal dosage range.

In the past, an antiamebic pharmaceutical preparation containing both clioquinol and phanquinone has been sold by CIBA GEIGY under the trademark Mexafor. However, the marketing of this preparation was stopped when it was supposed that clioquinol caused SMON.

Also phanquinone has received renewed interest in recent years and has been suggested for the treatment of Alzheimer's disease in WO 99/09981.

Disclosure of the invention

According to the present invention the new use of clioquinol for the manufacture of a pharmaceutical composition for the treatment or prevention of pathological conditions influenced by the action of MMP is provided.

Various diseases are influenced by MMPs. Examples of such diseases are tumor metastasis and neo-angiogenesis, including breast, colorectal, prostate, pancreatic cancer and leukemia; rheumatoid arthritis, osteoporosis and osteoarthritis; corneal ulceration; multiple sclerosis; diabetic complications, including periodontal disease; and atherosclerosis, including heart failure, myocardial infarction, and ischaemic heart disease. The common feature for the pathological conditions which may be influenced by MMPs is that such conditions involve tissue breakdown. In general, the cause of the disease influenced by MMPs is due to an over-activity of MMPs leading to increases degradation of tissue. However, in certain kinds of diseases, such as atherosclerosis, the lack of sufficient MMP activity may provide for growth of undesired tissues, such as atheromas.

The dosage of clioquinol optimal *in vivo* for treatment or prevention of the pathological condition influenced by MMPs may be determined by a physician upon conducting routine experiments. An example of such an experiment is to monitor the inhibiting effect of clioquinol in an extracellular body fluid in contact with the tissue affected by the pathological condition. Beginning with relatively low doses (5-10 mg/day), a physician may monitor the inhibition of the MMPs in the body fluid. If there is no or only an insubstantial increase in the inhibition of the MMPs, the dosage may be raised until such a desired inhibition is observed. Another example is monitoring the clinical signs and symptoms of the pathological condition by using clinical measurements.

The amount of clioquinol administered to a subject in need thereof must be sufficient to treat or prevent the pathological condition influenced by the action of MMPs. In one aspect of the invention, the daily administered amount of clioquinol is 1 mg to 1 g. E.g. the clioquinol may be administered in an amount of 5 mg to 100 mg one to three times daily. However, it may be desired to administrate clioquinol for some indications in amounts in excess of 1 g per day. According to another aspect of the invention, cliqouinol is administered in an amount of 1 g to 10 g per day.

As clioquinol is a chelator which scavenge heavy metals, it may be desired to administrate a metal salt or prosthetic group prior to, concurrent with or subsequent to the administration of clioquinol to avoid deficiency of said metal salt or prosthetic group. Previously, it has been demonstrated in WO 99/34807 that the re-uptake of the prosthetic group hydroxycobalamin (vitamin B_{12}) is inhibited or

prevented by clioquinol administering. In a preferred embodiment of the present invention it is therefore secured that the level of vitamin B_{12} in the subject being treated is sufficient for maintaining normal functions of the body. Preferably, vitamin B_{12} is administered together with clioquinol. The amount of vitamin B_{12} is suitably sufficient for impeding any detrimental side effect of clioquinol administration. A suitable daily amount of vitamin B_{12} is 5 µg to 2 mg. Preferably, the amount of vitamin B_{12} is 0.5 mg to 1 mg. It may be desired, in a first period to administrate clioquinol and in a second period the metal salt or prosthetic group. As an example, the first period may be one to three weeks and the second period one to four weeks.

It may be desired to administrate a further inhibitor of MMPs besides clioquinol. In a preferred embodiment of the invention, one or more further inhibitors of MMPs different from clioquinol is administered prior to, concurrent with or subsequent to the administrating of clioquinol, said further inhibitors having another activity toward the individual MMPs. The advantage of co-administration of one or more further inhibitors of MMPs is due to the fact that the MMP group consists of at least 13 different enzymes responding differently to a specific inhibitor. Administration of a further inhibitor besides clioquinol may allow for a targeted treatment of a certain pathological condition. Various inhibitors of MMPs are disclosed in the prior art and may be selected by the person skilled in the art according to the need thereof. The amount of the further inhibitor is preferably sufficient for increasing the effect of the prevention or treatment of the pathological condition influenced by the action

of MMP. A suitable daily amount of the further inhibitor may be 1 mg to 1 g, preferably 5 mg to 250 mg.

In a preferred embodiment of the present invention, the second or further inhibitors of MMPs is phanquinone. Thus, phanquinone may be administered prior to, concurrent with, or subsequent to the administering of clioquinol.

Phanquinone may be administered in any amount effective for treatment or prevention of the pathological disorder influenced by the action of MMPs. Notably, phanquinone may be administered in an amount of 5 mg to 250 mg one to three times daily.

According to an embodiment of the present invention phanquinone, clioquinol and vitamin B_{12} are used for the manufacture of the pharmaceutical composition.

The pharmaceutical composition manufactured using clioquinol may be formulated in any galenic formulation enabling clioquinol to enter the body. Generally, suitable formulations include pharmaceutical compositions formulated for oral, parenteral or intradermal administration.

Parenteral formulations include intravenous infusions and injection liquids. Parenteral formulations are generally preferred when high dosages are to be administered and in the treatment of acute disease states.

It may be desirable to formulate the pharmaceutical composition as a single pharmaceutical composition in cases wherein the pharmaceutical composition comprise more than one active component. Furthermore, including the active ingredients in a single pharmaceutical composition decreases the possibility of maltreatment of the subject. However,

it may be advantageous to formulate the pharmaceutical composition as two or more separate pharmaceutical entities for sequential or substantially simultaneous administration.

In one aspect of the invention a method is provided for the treatment of a subject having or suspected of having a pathological condition influenced by the action of MMP, comprising administering to the subject an amount of clioquinol effective to treat or prevent the pathological condition.

In another aspect of the invention a method is provided for treating a subject having or suspected of having a pathological condition influenced by the action of MMP, comprising administering to the subject an amount of clioquinol effective to inhibit the action of MMP.

In a further aspect of the invention a method is provided for treating a subject having or suspected of having a pathological condition influenced by the action of matrix metalloproteinase (MMP), comprising administering to said subject:

(a) an amount of clioquinol effective to treat or prevent the pathological condition influenced by the action of MMP, and

(b) an amount of a compound or a mixture of compounds selected from the group comprising metal salts, prosthetic groups and inhibitors of MMPs different from clioquinol having another activity towards the individual MMPs.

Detailed description of the invention

In the following the invention will be explained in further detail. The proposed mechanism of action of

the invention is not intended to limit the invention to said mechanism.

At present, the applicant believes that clioquinol and matrix metalloproteinases by competition chelate zinc from a common pool. Clioquinol has the ability to penetrate tissues, biological fluids, cells and pathological formations like atheromas, metastatic cells, degenerative cells, neo angiogenesis cells and inflammatory tissue. When clioquinol has entered the biological area involved in the pathological condition, the zinc(II) ion is captured from the free pool existing due to the equilibrium between MMPs containing zinc and MMPs lacking zinc. Clioquinol having chelated a zinc(II) ion then moves away from the area involved in the pathological condition and into the interstitial fluid, the lymph, the blood, the urine or the bile and is cleared from the body. The deprivation of zinc from the direct environment of the zinc requiring matrix metalloproteinases inhibits the action of the MMPs.

The pharmaceutical composition manufactured using clioquinol preferably comprises one or more pharmaceutical acceptable carriers and, optionally, one or more further active constituent(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipients thereof. In a preferred embodiment, the clioquinol and, optionally, further active constituents in the pharmaceutical composition are purified.

It will be appreciated that the amount of clioquinol and, optionally, further active constituents required for said treatment or prevention will vary according to the route of administration, the disorder to be treated, the condition, age, the

file history of the subject, and the galenic formulation of the pharmaceutical composition, etc. When treating a patient diagnosed as having a pathological condition influenced by the action of MMPs, the amount of clioquinol is preferably effective to provide for at least a partially inhibition of at least one of the enzymes belonging to the group of MMPs.

In one aspect of the invention, a suitable therapeutically effective amount of clioquinol in the pharmaceutical composition is, for example, 1 mg to 1 g, preferably 5 mg to 100 mg. In another aspect of the invention, up to 10 g of clioquinol may be formulated in a single pharmaceutical composition. If phanquinone and vitamin B_{12} are selected as further active ingredient of the pharmaceutical composition, the amount of phanquinone is preferably effective to the treatment or prevention of the pathological disorder influenced by the action of MMPs, and the amount of vitamin B_{12} is preferably effective to inhibit a detrimental side effect of clioquinol administration. The amounts of phanquinone and vitamin B_{12} are preferably 5 mg to 250 mg, more preferred 10 mg to 50 mg and 5µ to 2 mg, most preferred 0.5 mg to 1 mg, respectively.

The actually administered amounts of clioquinol and, optionally, further active constituents, such as phanquinone and vitamin B_{12}, may be decided by a supervising physician. If the pharmaceutical composition in addition to clioquinol comprises further active constituents they may be in the same composition for administering in combination concurrently, or in different compositions for administering substantially simultaneously but separately, or sequentially. If the active

constituents are administered sequentially, the further active ingredients may be administered prior or subsequently to the administering of clioquinol.

Pharmaceutical formulations include those suitable for parenteral (including intramuscular, intracoronary, intra-articular and intravenous), oral, rectal or intradermal administration. Oral administration is the preferred route in one aspect of the invention, while the parenteral route is preferred in another aspect of the invention. Thus, the pharmaceutical composition may be formulated as tablets, pills, syrups, capsules, suppositories, solutions or emulsions for parenteral injection or infusion, formulations for transdermal application, powders, especially lyophilized powders for reconstitution with a carrier for intravenous administration, etc. The pharmaceutical compositions may be prepared using conventional carriers.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapy is administered. The carriers in the pharmaceutical composition may comprise a binder, such as microcrystalline cellulose, carboxymethylcellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatine, starch, lactose or lactose monohydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate, or sodium lauryl sulphate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and/or a flavouring agent, such as peppermint, methyl salicylate, or orange flavouring.

Pharmaceutical formulations suitable for oral administration, e.g. tablets and pills, may be obtained by compression or moulding, optionally with

one or more accessory ingredients. Compressed tablets may be prepared by mixing the constituent(s), and compressing the mixture obtained in a suitable apparatus into tablets having a suitable size. Prior to the mixing, the clioquinol may be mixed with a binder, a lubricant, an inert diluent and/or a disintegrating agent and the further optionally present constituents may be mixed with a diluent, a lubricant and/or a surfactant.

In a preferred embodiment, free-flowing clioquinol powder is mixed with a binder, such as microcrystalline cellulose, and a surfactant, such as sodium lauryl sulphate, until a homogeneous mixture is obtained. Subsequently, another binder, such as polyvidone, is transferred to the mixture under stirring. When a uniform distribution is obtained an aqueous solution of vitamin B_{12} is added under constant stirring. Said mixture is passed through granulating sieves and dried by desiccation before being compressed into tablets in a standard compressing apparatus.

In a second preferred embodiment, free-flowing clioquinol powder is mixed with surfactants and/or emulsifying agents, such as Sapamine® (N-(4'-stearoyl amino phenyl)-trimethylammonium methyl sulphuric acid) and lactose monohydrate until a uniform distribution of the constituents is obtained. A second preparation containing a disintegrating agent, such as maize starch, is added to the clioquinol mixture while being continuously stirred. Such a second preparation may be prepared by adding excess boiling water to a maize starch suspended in cold water. The final mixture is granulated and dried as above and mixed with maize starch and magnesium stearate and finally compressed into tablets in a standard apparatus.

A tablet may be coated or uncoated. An uncoated tablet may be scored. A coated tablet may be coated with sugar, shellac, film or other enteric coating agents.

Pharmaceutical formulations suitable for parenteral administration include sterile solutions or suspensions of the active constituents. An aqueous or oily carrier may be used. Such pharmaceutical carriers may be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Aqueous parenteral solutions for intravenous or intra-articular injection or infusion may be prepared by dilution to the desired concentration with an aqueous solvent or emulsifying agent, like water containing dissolved carboxymethylcellulose or polysorbate, such as polysorbate 80, ethyl oleate, Tween 20, or the like. Prior to the dissolution, clioquinol may initially be pre-dissolved in an organic solvent, preferably an aprotic solvent like DMSO, DMF, and the like. Formulations for parenteral administration also include a lyophilized powder comprising clioquinol and, optionally, further active constituents that is to be reconstituted by dissolving in a pharmaceutically acceptable carrier that dissolves the active constituents, e.g. an aqueous solution of carboxymethylcellulose and lauryl sulphate. Parental formulations are preferably made isotonic by adjusting with suitable electrolytes.

When the pharmaceutical composition is a capsule, it may contain a liquid carrier, such as a fatty oil, e.g. cacao butter.

Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt,

rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The compositions may be solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition may be formulated as a suppository, with traditional binders and carriers such as triglycerides.

In yet another embodiment, the clioquinol may be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14: 201 (1987); Buchwald et al., Surgery 88: 507 (1980); Saudek et al., N. Engl. J. Med. 321: 574 (1989)). In another embodiment, polymeric materials may be used (cf. Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23: 61 (1983); see also Levy et al., Science 228: 190 (1985); During et al., Ann. neurol. 25: 351 (1989); Howard et al., J. Neurosurg. 71: 105 (1989)). In yet another embodiment, a controlled release system may be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (cf. e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249: 1527-1533 (1990)).

In one embodiment of the pharmaceutical composition, clioquinol and the, optionally, further active constituents, are comprised as separate

pharmaceutical entities. By way of example, one entity may comprise clioquinol and another entity may comprise vitamin B_{12}. The two entities, may be administered simultaneously or sequentially. For example, the entity comprising clioquinol can be administered, followed by vitamin B_{12} administered within a day, week, or month of clioquinol administration. If the two entities are administered sequentially, the entity comprising clioquinol is preferably administered for one to three weeks followed by a wash out period of one to four weeks, during which the entity comprising vitamin B_{12} is administered but not the entity comprising clioquinol. After the wash out period, the treatment may be repeated.

The pharmaceutical composition may be provided as a pack or kit comprising one or more entities containing one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally, associated with such entities may be a notice in the form described by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The various different diseases influenced by the action of MMPs which may be treated according to the present invention can be administered clioquinol and optionally further pharmaceutical active compounds in accordance with suitable dosage forms and regimes. As an example, neoplasias, such as neo-angiogenesis, tumors, and neoplastic diseases, may be treated by infusing 0.5 g to 5 g clioquinol, preferably about 1 g, dissolved or emulsified in a suitable amount of carrier, such as 100 ml to 1000ml, preferably around

250 ml for 1 to 4 weeks. The treatment may be repeated after 1 to 4 months if considered suitable by the attending physician. For solid tumors and advanced states of neoplasias the amount of clioquinol administered is generally in the higher end of the above range, that is between 1 g and 5 g. Between treatments with clioquinol by infusion, clioquinol may be administered orally, e.g. by administering 100 mg to 1 g one to three times daily.

Another example is the treatment of rheumatic diseases, such as osteo-arthritis, rheumatoid arthritis, and autoimmune diseases. Suitably, these diseases may be treated by intra-articular injection or infusion of 500 to 1000 mg clioquinol dissolved in an appropriate amount and kind of carrier in a time period of 4 to 14 days. Alternatively, the same dosage regime as described for neoplasias may be used.

Yet another example is the treatment of acute coronary syndromes, such as unstable angina, refractory unstable angina and acute myocardial infarct. Acute coronary syndromes may be treated by administering 500 mg to 5 g clioquinol dissolved in an appropriate amount and kind of solvent. Suitably, the mode of administration can be intra-coronary or intravenous infusion during the acute phase of the disease. For refractory unstable angina or for large infracts or for highly thrombogenic coronary arteries, the amount of clioquinol is usually in the higher end of the above range, i.e. between 1 g and 5 g. Optionally, the acute phase treatment can be followed by orally administration of clioquinol in an amount of 100 mg to 1g, preferably about 250 mg, one to three tomes daily for 1 to 8 weeks.

Other features and advantages of the invention will be apparent from the following examples, which,

in conjunction with the accompanying drawings, illustrate by way of example the principles of the invention.

Examples

EXAMPLE 1

Preparation of a pharmaceutical composition comprising clioquinol

250 g of clioquinol were mixed with 200 g sapamine® (N-(4'-stearoyl amino-phenyl)-trimethylammonium methyl sulphuric acid) and 1025 g lactose mono-hydrate for a period of 5 minutes. 300 g of boiling water was added in one go to a mixture of 100 g maize starch in 100 g cold water. The maize suspension, cooled to 40°C, was added to the clioquinol-containing powder mixture under continuous stirring. The mixture was granulated using a 2.5 mm sieve and desiccated for 18 hours at 40°C. The dry granules were mixed with 400 g maize starch and 20 g magnesium stearate. The final mixture was formulated into tablets having a diameter of 8.0 mm and a weight of 200 mg.

EXAMPLE 2

Preparation of a pharmaceutical composition comprising clioquinol and vitamin B_{12}

250 g of clioquinol (5-chloro-7-iodo-8-quinoline) were mixed with 200 g sapamine® (N-(4'-stearoyl amino-phenyl)-trimethylammonium methyl sulphuric acid) and 1025 g lactose mono-hydrate for a period of 5 minutes. 300 g of boiling water was added in one go to a mixture of 100 g maize starch in 100 g cold water. The maize suspension, cooled to 40°C was added to the

clioquinol- containing powder mixture under continuous stirring. Subsequently, an aqueous solution of 5 g vitamin B_{12} was added. The mixture was granulated using a 2.5 mm sieve and desiccated for 18 hours at 40°C. The dry granules were mixed with 400 g maize starch and 20 g magnesium stearate. The final mixture was formulated into tablets having a diameter of 8.0 mm and a weight of 200 mg.

EXAMPLE 3

Preparation of a pharmaceutical composition comprising phanquinone, clioquinol and vitamin B_{12}

250 g phanquinone and 250 g of clioquinol were mixed with 200 g sapamine® (N-(4'-stearoyl amino-phenyl)-trimethylammonium methyl sulphuric acid) and 1025 g lactose mono-hydrate for a period of 5 minutes. 300 g of boiling water was added in one go to a mixture of 100 g maize starch in 100 g cold water. The maize suspension, cooled to 40°C was added to the phanquinone and clioquinol containing powder mixture under continuous stirring. Subsequently, an aqueous solution of 5 g vitamin B_{12} was added. The mixture was granulated using a 2.5 mm sieve and desiccated for 18 hours at 40°C. The dry granules were mixed with 400 g maize starch and 20 g magnesium stearate. The final mixture was formulated into tablets having a diameter of 8.0 mm and a weight of 200 mg.

EXAMPLE 4

Inhibition study

An enzyme assay was conducted with five of the enzymes belonging to the MMP group. Specifically, the assay was conducted for MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9 at various concentrations.

The MMP-1, MMP-3, and MMP-7 were initially pre-incubated in 60 min at 37°C and MMP-2 and MMP-9 were pre-incubated in 60 min at 25°C in an aqueous vehicle of 50 mM MOPS, 10mM $CaCl_2.2H_2O$, 10 µM $ZnCl_2$, 0,05% Brij 35, pH 7.2 and a concentration of cliqouinol of 100 µM. A test substrate of Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 was subsequently added to obtain a concentration of 25 µM. MMP-1 was incubated for 2 hours at 37°C, MMP-2 was incubated for 3 hours at 25°C, MMP-3 was incubated for 90 min at 37°C. MMP-7 was incubated for 90 min at 37°C, and MMP-9 was incubated for 2 hours at 25°C. The activity of the enzymes was measured by fluorometric quantitation of Mca-Pro-Leu-Gly-OH. The results are indicated in Table I below.

Table I

MMP enzyme	% Inhibition
MMP-1	12
MMP-2	28
MMP-3	7
MMP-7	20
MMP-9	19

EXAMPLE 5

Inhibition study for high dosages

The enzyme assay shown in example 4 was repeated for MMP-2 except that a 10 and 100 times higher clioquinol concentration was used. Thus, at a clioquinol concentration of 1 mM the inhibition was 26% and at a clioquinol concentration of 10mM the inhibition was measured to 101%.

The results indicate that the inhibition is highly dependant on the clioquinol concentration.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

It will be obvious to a person skilled in the art that the invention thus described may be varied in many ways. Such variation are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications, as would be obvious to a person skilled in the art, are intended to be included in the scope of the following claims.

CLAIMS

1. A use of clioquinol for the manufacture of a pharmaceutical composition for treatment or prevention of pathological conditions influenced by the action of matrix metalloproteinase (MMP).

2. The use according to claim 1, wherein the disease influenced by the action of matrix metalloproteinase is tumor metastasis and neo-angiogenesis, including breast, colorectal, prostate, pancreatic cancer and leukemia; rheumatoid arthritis, osteoporosis and osteoarthritis; corneal ulceration; multiple sclerosis; diabetic complications, including periodontal disease; and atherosclerosis, including heart failure, myocardial infarction, and ischaemic heart disease.

3. The use according to claim 1 to 2, wherein clioquinol is administered in a daily amount of 1 mg to 1 g.

4. The use according to claim 1 or 2, wherein clioquinol is administered in a daily amount of 1 g to 10 g.

5. The use according to any of the preceding claims, wherein a metal salt or prosthetic group is administered prior to, concurrent with, or subsequent to the administering of clioquinol.

6. The use according to claim 5, wherein the protetic group is vitamin B_{12}.

7. The use according to any of the preceding claims, wherein the amount of vitamin B_{12} is effective to inhibit a detrimental side effect of clioquinol administration.

8. The use according to claim 7, wherein the amount of vitamin B_{12} is 5 μg to 2 mg.

9. The use according to claim 7, wherein the amount of vitamin B_{12} is 0.5 mg to 1 mg.

10. The use according to any of the preceding claims, wherein an inhibitor of MMPs different from clioquinol and having another activity towards the individual MMPs is administered prior to, concurrent with or subsequent to the administering of clioquinol.

11. The use according to claim 10, wherein the inhibitor different from clioquinol and having another activity is phanquinone.

12. The use according to claim 11, wherein phanquinone is administered in an amount of 5 mg to 250 mg one to three times daily.

13. The use according to any of the claims 10 to 12, wherein phanquinone is administered in an amount of 10 mg to 50 mg one to three times daily.

14. The use according to any of the claims 6 to 13, wherein phanquinone, clioquinol and vitamin B_{12} are used for the manufacture of the pharmaceutical composition.

15. The use according to any of the preceding claims, wherein the pharmaceutical composition is formulated for oral, parenteral or intradermal administration.

16. The use according to any of the claims 1 to 15, wherein the pharmaceutical composition is formulated as a single pharmaceutical composition.

17. The use according to any of the claims 6 to 16, wherein the pharmaceutical composition is formulated as two or more separate pharmaceutical entities for sequential or substantially simultaneous administration.

18. A method of treating a subject having or suspected of having a pathological condition influenced by the action of matrix metalloproteinase (MMP), comprising administering to the subject an

amount of clioquinol effective to treat or prevent the pathological condition.

19. The method according to claim 18, wherein the disease influenced by the action of matrix metalloproteinase is tumor metastasis and neo-angiogenesis, including breast, colorectal, prostate, pancreatic cancer and leukemia; rheumatoid arthritis, osteoporosis and osteoarthritis; corneal ulceration; multiple sclerosis; diabetic complications, including periodontal disease; and atherosclerosis, including heart failure, myocardial infarction, and ischaemic heart disease.

20. A method of treating a subject having or suspected of having a pathological condition influenced by the action of matrix metalloproteinase (MMP), comprising administering to the subject an amount of clioquinol effective to inhibit the action of MMP.

21. A method of treating a subject having or suspected of having a pathological condition influenced by the action of matrix metalloproteinase (MMP), comprising administering to said subject:

(a) an amount of clioquinol effective to treat or prevent the pathological condition influenced by the action of MMP, and

(b) an amount of a compound or a mixture of compounds selected from the group comprising metal salts or prosthetic groups and inhibitors of MMPs different from clioquinol having another activity towards the individual MMPs.

22. The method according to claim 21, wherein the total amount of the compound(s) in (b) is sufficient for increasing the effect of the prevention or treatment of a pathological condition influenced by

the action of MMP or for impeding any detrimental side effect.

23. The method according to claim 18, 20, or 21, wherein the daily administered amount of clioquinol is 1 mg to 1 g.

24. The method according to claim 18, 20, or 21, wherein the daily administered amount of clioquinol is 1 g to 10 g.

25. The method according to claim 21, wherein the amount of the compound(s) in (b) is 5 µg to 250 mg.

26. The method according claim 21, wherein the inhibitor different from clioquinol and having another activity is phanquinone.

27. The method according to claim 21, wherein the prosthetic group is vitamin B_{12}.

28. The method according to claim 21, wherein the amount of vitamin B_{12} is 5 µg to 2 mg.

29. The method according to claim 21, wherein the amount of vitamin B_{12} is 0,5 mg to 1 mg.

30. A method of treating a subject having or suspected of having pathological conditions influenced by the action of matrix metalloproteinase (MMP) comprising administering to the subject:

(a) an amount of clioquinol effective to treat or prevent the action of MMP, and

(b) a mixture of phanquinone and vitamin B_{12}, the amount of phanquinone being effective to treat or prevent pathological conditions influenced by the action of MMP and the amount of vitamin B_{12} being effective to inhibit a detrimental side effect of clioquinol administration.

31. The method according to claim 21 or 30, wherein (a) clioquinol and (b) the compound(s) are comprised in a single pharmaceutical composition.

32. The method according to claim 21 or 30, wherein (a) clioquinol and (b) the compound(s) are administered substantially simultaneously.

33. The method according to claim 21 and 30, wherein (a) clioquinol and (b) the compound(s) are administered sequentially.

34. The method according to claim 21 or 30, wherein clioquinol and vitamin B_{12} are administered sequentially.

35. The method according to claim 21 or 30, wherein clioquinol is administered in a first period followed by a second period, wherein vitamin B_{12} is administered.

36. The method according to claim 35, wherein the first period is one to three weeks and the second period is one to four weeks.

37. The method according to any of the claims 18 to 36, wherein the subject is human.

38. The method according to claim 18, 21 or 30, wherein clioquinol is formulated for oral administration.

39. The method according to claim 18, 21 or 30, wherein clioquinol is formulated for parenteral administration.

40. The method according to claim 18, 21 or 30, wherein clioquinol is formulated for intradermal administration.